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A BRIEF CHANGE IN HOUSING CONDITIONS ALTERS THE SURVIVABILITY OF IRRADIATED C3H/HeN MICE.

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ABSTRACT

The present experiment examined how 24-hours in different housing conditions could effect the longevity of male and female C3H/HeN mice exposed to 8.0, 8.4, and 8.8 Gy ^{60}Co radiation at a dose rate of 0.40 Gy/minute. Housing conditions had a significant effect on the longevity of mice, as did gender and Gy level. Normal housed male and female mice (i.e., 10 mice per large 15800 cm^3 cage) lived longer than did mice housed in isolation (i.e., one mouse per similar cage). Female mice exposed to 8.0 Gy and housed in either a social crowded (i.e., 10 mice per small 1580 cm^3 cage) condition or a social and spatial crowded (i.e., 1 mouse per 158 cm^3 cage -- placed side-by-side in a 2x5 matrix) condition lived longer than did either normal or isolated female mice. The housing effect was so pronounced for female mice that 30% of the mice in the social crowded condition and 60% of the mice in the social and spatial crowded condition survived the supra-lethal 8.0 Gy dose. Female mice in the social and spatial condition lived 34% longer than did female mice housed in isolation. Overall, female mice lived 20% (+/- 5%) longer than did male mice.

Male mice housed in the social crowded condition lived on the average 23% (+/- 7%) longer than male mice housed in isolation. Male mice housed in the spatial crowded condition lived on the average 18% (+/- 7%) longer than male mice housed in isolation. Male mice in the 8.0 or 8.4 Gy condition lived an average of 35% longer (+/- 5%) longer than male mice in the 8.8 Gy condition.

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Male mice housed in the social crowded condition lived on the average 23% (+/- 7%) longer than male mice housed in isolation. Male mice housed in the spatial crowded condition lived on the average 18% (+/- 7%) longer than male mice housed in isolation. Male mice in the 8.0 or 8.4 Gy condition lived an average of 35% longer (+/- 5%) longer than male mice in the 8.8 Gy condition.

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This study examined 5 housing conditions and 3 radiation dose levels. The results of this study demonstrate gender, housing, and dose effects. Female mice lived longer than male mice, lower Gy levels were associated with increased longevity, and housing conditions influenced the radioresistance of both male and female mice. Enhanced radioresistance and enhanced radiosensitivity was associated with certain housing conditions.

Key Words:

Longevity
Enhanced Radioresistance
Crowding
Isolation

INTRODUCTION

This study examined the survivability of C3H/HeN mice housed in different ways after exposure to ionizing radiation. Mice were held in several distinct housing conditions for 24-hours after exposure to ^{60} Co gamma photons. A 8.0 Gy supra-lethal Gy exposure of ^{60} Co was not lethal for 60% of social and spatial crowded mice (n=1 mouse/158 cm^3cage placed side by side in a 2x5 matrix, See Figure 1) or 30% of social crowded (n=10 mice/1575 cm^3cage) female mice. In addition, mice housed in a social crowded condition (n=10 mice/1575 cm^3cage) lived 30% longer than isolated mice (n=1 mouse/16573 cm^3cage).

According to a 1987 National Research Council (NRC) report[1] on space biology and medical science habitability issues have been and will continue to be important for successful space travel. The NRC report states that living quarters will be crowded during space exploration. Consequently, the possibility of long-term occupancy of space vehicles by humans will depend on the effective design of space habitats. Specific high priority research areas that were identified by the NRC report includes environmental factors, such as the influence of variations of architecture during occupancy, stressful conditions (and their amelioration), and sensory deprivation.

Environmental factors, such as crowding[2,3,4], isolation[5,6,7], and ionizing radiation[8] are all recognized as stressors that normally occur in nature in varying degrees. Typically, environmental stressors such as crowding or isolation require the organism to perceive the situation as being stressful before the cascade effects of the stress response are activated[9]. Ionizing radiation is a unique environmental stressor. Irradiation can occur without the organism being initially aware of it or of the immunosuppressive, hematopoietic insult and collateral tissue damage associated with this physical toxin. Of interest to medical personnel, scientists, astronauts, and submariners is the linkage between altered housing conditions, immune functioning and the hematopoietic cells. Of further interest to these professionals is that exposure to ionizing radiation also has a damaging effect on the hematopoietic system. The combined stress of an adversive housing condition and exposure to ionizing radiation could suggest, that

decreased longevity might occur, if mice were exposed to these multiple stressors. This study examined how housing conditions alter the radioresistance (survivability of) C3H/HeN mice.

Gamma Radiation

Previous investigations regarding the concomitant influence of certain environmental factors on the longevity of rodents exposed to Cobalt-60 gamma radiation dates back to the late-1950's and mid-1960s. Kirby[10] demonstrated that if isolation was carried out for a prolonged period it resulted in profound psychological and physiological changes. Grahn et al.,[11] examined the influence of cobalt-60 radiation, environmental factors, and gender on life shortening in C3H/He mice. Subjects were followed throughout their entire lifespan while they were being exposed to radiation. These life-time studies of radiation injury indicated an unusual sensitivity to the cage environment expressed as a modification of life expectancy. The investigators examined mice of both genders, housed in either an isolated (n=1) or a crowded (n=3) cage (i.e., cage size was 50 cm^3). Longevity results indicated statistically significant dose and cage effects along with a small gender effect. Specifically, female mice outlived males, control mice outlived experimental mice, and crowded mice (i.e., trios) outlived isolated mice.

Sacher and Grahn[12] extended this work and reported on the importance of environmental factors in survival studies, emphasizing that small differences in housing parameters can influence survival outcomes. Other research by Lesher[13] used 100-day old C57 (LAF1) mice. His mice were housed three to a cage. Sex differences in survival time following life-time exposure to the same amount of gamma radiation, were also noted, with female mice surviving longer than male mice. Previously, a review of the radioresistance of females was reported by Grahn[14] across several inbred mouse strains. Sacher and Grahn[12] found that at low doses and dose rates, the sex difference tended to first appear at about 18 days, and be maximal between 25 and 30 days after exposure, in all the strains they had studied.

Vogel et al.,[15] used 100 day old CF#1 female mice. The mice weighed between 20 and 28 grams, were housed 8 per cage (i.e., each mouse had access

to 1000 sq. cm. of space), and exposed them to gamma radiation (i.e., dose rate between 8-12 r/min and a total dose of 8.1, 9.4, and 10.4 Gy) and found that the mean survival time was 357.6 hours (i.e., 14.9 days) for the lower total dose and 326.4 hours (i.e., 13.6 days) for the two higher doses. Ainsworth et al.,[16] used 3-4 month old CF#1 female mice weighing between 22-28 grams, housed 10-12/cage (i.e., no dimensions were provided for the housing condition), and exposed them to Co-60 gamma radiation (i.e., 775-821 rads) at a dose rate of 100 rads a minute. He found that mean survival times of these normally housed mice were 302.4 hours (i.e., 12.6 days). However, different times to death would be expected between these two studies given the different biological effects associated with the varied doses, dose rates, and housing conditions.

More recent literature addressing the importance of housing conditions and environmental factors can be found in the Guide for the Care and Use of Laboratory Animals[17] manual published by the National Institute for Health, the Jackson Laboratory handbook[18] on genetically standardized mice, and a book by Festing[19] that reviews the unique susceptibility of species to environmental alterations. These documents are consistent with previous research and all concur that small alterations of environmental factors can influence experimental outcomes. Specifically, altered housing may result in different experimental findings across laboratories and across (or within) species.

CROWDING

Laboratory research, using animals, has demonstrated that environmental stressors can alter immune function[20,21,22]. Depending upon the intensity and the context in which environmental stressors (e.g., isolation or crowding) occur, can ameliorate or exacerbate the organism's stress response[23,24]. This research supports the importance of housing conditions in the maintenance of the health of rodents and other animals. Several animal studies [25,26,27] have altered housing conditions in an attempt to determine longevity differences. An early study by Andervont[28] housed female mice with a genetic predisposition for mammary tumors in either isolated, or group (i.e., 8/cage) conditions. He found that isolated housing resulted in decreased survivability (mean = 9.6 months) compared to group housed mice (mean = 11.9 months). He also reported that ninety-eight percent of the isolated mice developed mammary tumors, whereas

only eighty-percent of the group housed animals developed tumors. These empirical findings suggest that the manipulation of housing mice in isolation impeded their immune functioning (i.e., enhanced tumor development) and reduced longevity.

Edwards et al,[25] has also demonstrated that mice housed in continuous isolation had significantly higher antibody levels than group housed mice or mice transferred from an isolated environment into a group housed environment. Boranic et al.,[29] examined the immune responses to mice placed for one week in an overcrowded (n=20/cage) condition or a group housed (n=5-6/cage) condition. Immune responses were measured by colony formation of bone marrow stem cells. The overcrowded mice had suppression of colony counts in vitro. These results suggest differential housing, effects the generation of hematopoietic cells. Research by Rabin[27] has extended our knowledge regarding the responsivity of the immune system to environmental stressors, such as altered housing conditions. Rabin[30] found that the C3H/HeJ male mouse, when changed from group housing (n=5) to one animal per cage, had enhanced T cell related immune function. Female C3H/HEJ mice did not show this effect. Rabin also showed that individually housed male animals were significantly more resistant to infection than group housed (n=5) mice. Specifically, mice in the isolated condition required approximately 2.5 times more of the Candida Albicans organism to infect 50% of the kidneys compared to the group housed mice (n=5). The time course of this enhanced resistance (to pathogens) is present approximately 10 days after being placed in isolation (i.e., 1 per cage) and lasts approximately 3 weeks[30]. He concludes that the immune system responsiveness appears to be related not only to the number of mice housed per cage (i.e., 1 vs 5), but also to gender and other environmental factors. Landauer[31] used C57 mice and found that group housed irradiated mice did not survive as long as irradiated isolated mice. However, this mouse strain is known for its aggressive combatant tendencies when it is group housed with others (Jackson Laboratory, 1988). Thus, deaths due to combat are likely to have contributed to his findings.

Coe et al.,[32,33] showed that levels of antibodies circulating in the bloodstream, generally thought to change only in response to immunizations, disease or drug treatment, can be strongly influenced by housing conditions, such

as isolating infant monkeys from their mothers (In Press). Coe[34] studied the antibody response to viral challenge in 6-month old monkeys that had been removed from their mothers for 7-day periods in different separation environments. He demonstrated that monkeys housed individually in unfamiliar cages showed significantly reduced antibody levels. However, those monkeys that were housed with three or four familiar companions in their home cages mounted normal antibody responses. Thus, environmental factors, such as housing conditions also affect other species, including primates.

Laboratory experiments[35], field studies[36,37], and operational experience[38,39], have all shown that altered housing conditions affect human behaviors[1,40,41], perceptions[9,40,42], neuroendocrine functioning[43,44], and immune system performance[6,44]. Results of altered housing conditions from submarine cruises consisting of a 40-man crew showed a "definite increase in feelings described as irritability, annoyed, feel like giving up, bored stiff, uncomfortable, and frustrated[38]. Other studies from Polaris submarine 60-day missions reported that submarine personnel were treated for depression, headaches, and insomnia. The crew also viewed their lack of privacy, from crowded conditions as a problem and reacted by using vulgar language, joking, and establishing pecking orders[41,45,46]. Simulation studies have found that prolonged isolation causes increased irritability and hostility with accompanying decrements in psychomotor skill performance, memory, judgement, and learning abilities[40].

Recently, Stefania Follin, a 27-year old woman spent a record 130 days isolated in a New Mexico cave. The major findings of this study were that she lost track of time, worked approximately 22-hour days, experienced a weight loss of 17 pounds, and a cessation of menses[47]. In addition, during the weeks of her stay, she experienced bouts of irritability, and periods of withdrawal from her daily regime. Other isolation studies report periods of depression, mood swings and irritability associated with crowded conditions[1,7,42,48]. Parker et al.,[49] reported that a reduction in crowding (i.e., more volume per man) is needed as a function both of increasing time and increasing crew size. If optimum habitability is considered the goal, design requirements for long-duration missions should be based on 600-700 cubic ft/man, with 350-400 cubic ft/man considered adequate[49]. Stuster[50] further describes that the space available for sleeping should be aproximately 84 cubic feet (e.g., 3'x4'x7'), while Taylor[51]

offers a modular approach to managing the crowded conditions on future space flights.

A subjective report of what cosmonauts think about working in crowded conditions aboard the Soviet space station Mir have implications for future space travel. A UPI news release[52] reports that Vladimir Shatalov, a former cosmonaut and now Chief of cosmonaut training said the next manned mission to the Mir will depend upon when one, of two, new promised modules will be ready to relieve crowding on the station. He reports, "It's hard to live up there in the station now because it is stuffed with equipment". Ten years prior[53] to this press release, two cosmonauts, Popovich and Artillkhin reviewed the space craft designs of the Vostok, Soyuz, and Salyut space capsules in which they had flown. They concluded that increases in cabin space (i.e., a reduction of crowding--social and spatial) were necessary in order to accommodate more complex missions and to enhance social and psychological harmony between cosmonauts. Thus, numerous authors[49,50,51,52,53,54] concur on the importance of space station habitability issues (i.e. space station design) in the maintenance of social harmony during long-duration space flights.

Another example of how housing conditions are important to a human can be found from an accident at a waste treatment facility located near Richland, WA[55]. A human male was seriously contaminated with ^{241}Am while recovering waste material. During his recovery it was revealed that housing considerations were "very" important aspects of his treatment regime. The post-incident care and rehabilitation process associated with accidents involving irradiation involve an unusual degree of emotional trauma [56]. Brown emphasized the importance of ensuring that the patient was not housed in isolation. In cases of radionuclide contamination, it may not be possible for physical touching to occur immediately following the accident. However, the psychosocial comfort to the patient that accompanied the mere presence (i.e., sight and sound) of family members was an important factor in his initial recovery[55]. As the patient recovered, it was deemed necessary to move the patient out of the sterile clinical housing environment into a transition house trailer located next to the clinic. The patient, visitors and friends, and the family dog, occupied this facility to avoid the isolation stress associated with the sterility of the clinical setting. The enriched (i.e., nonisolated) housing environment was deemed an important part of

the patient's recovery by members of the treatment team, including the psychologist, medical, and radiation-monitoring personnel (Brown, 1983).

Future work is required to assess the short-term and long-term immune system differences reported, across genders, of isolated or group housed animals, with or without irradiation. Mouse studies using gamma radiation have traditionally measured time to death differences that resulted from different manipulations of doses or dose rates, different ages, genders, species, and one or two different housing conditions. What is needed is a study to demonstrate how dramatic the longevity effects may be from similarly handled mice exposed to several altered housing conditions. By studying the mortality of irradiated mice in different housing and dose conditions, we may overcome some of the extrapolation difficulties associated with attempting to generalize from the results of several previous studies that used a multitude of different end points[10,11,12,13,14,15,16,20,22,27,28,29,30]. The gamma irradiation studies cited earlier have examined the environmental stressors of isolation and group housing. The preponderance of the published literature strongly suggests that being housed in isolation results in quicker mortality than occurs in group housed animals. Previous longevity research has only addressed varying degrees of either "isolation" and/or "normally" housed animals, across numerous species (and strains) without achieving equivocal consensus. The following experiment sought to systematically investigate the differences in survival time of male and female C3H/HeN mice exposed to five different housing conditions exposed to three doses of supra-lethal radiation.

METHODS

In the present study we measured the time to death of irradiated mice in five different housing conditions. The housing conditions were: **isolated** (n=1), **normal housed** (n=10/cage), **social crowded** (n=10/small cage), **spatial** crowded, (n=1/very small cage), or **social and spatial** crowded (n=1 mouse per very small cage placed flush against each other in a 2 x 5 matrix (see Figure 1). **Spatial crowding** refers to a condition of being constrained due to physical boundries (i.e., very little available space) vs **social crowding** that

results from an over abundance of animals per unit area. Mice in each housing condition were exposed to one dose of supra-lethal Co-60 radiation (i.e., 8.0, 8.4, or 8.8 Gy) administered at a dose rate of 0.40 Gy/min. The procedural sequence of this experiment was: approximately 8-week old male and female mice were irradiated in well ventilated acrylic containers. Mice were then immediately transferred to their respective housing containers, following a quasi-random (i.e., mice were not rehoused with mice from their pre-experimental housing group) procedure, where they remained for 24-hours. In all cases, mice were never returned to their home cages and were always segregated by sex. Every housing container used in this experiment was well ventilated and had food, bedding, and acidified water (pH 2.5) available **ad libitum**. Mice were housed in an AALAC-approved animal care facility and were kept on a 12 hour day: 12 hour night cycle with no twilight periods. The temperature in their room was a consistent 21 degrees centigrade (+/- 1 degree), the humidity was 60% +/- 10 %, and the air was changed approximately 10 times every hour.

Following the 24-hour housing manipulations, mice were transferred to a standard, commercially available, polycarbonate mouse housing container (45.5 cms long x 23.5 cms wide x 15.5 cms in height), where they were housed 10 mice/cage for the next 29 days (or until they died). In order to be consistent with other longevity studies, mice in the **isolated** condition, were housed individually in the standard sized containers, following their 24-hour residence in the smaller **isolation** (i.e., **spatial crowded**) container. The hard-wood bedding in all of the mouse cages was changed twice weekly. Mice were all handled the same number of times across all the housing conditions. Consistent with historically established procedures, mortality measures were recorded twice daily[11,12].

Upon arrival, mice were housed in groups of ten using a commercially available plexiglass mouse container, quarantined for 2-weeks, and examined for serology, and histological anomalies, while acclimatizing to their surroundings. Mice were housed on hardwood bedding. A commercial well-balanced mouse chow and acidified pH 2.5 water was available <u>ad libitum</u>. Acidified water has been shown to be effective in inhibiting the multiplication of <u>Pseudomonas Aeruginosa</u>, the bacteria responsible for the early mortalities following irradiation of rodents harboring this organism.

The U.S. Department of Health and Human Services **Guide for the Care and Use of Laboratory Animals** (1985) recognizes that; "Special housing provisions are sometimes necessary for unusual laboratory species such as those with unique metabolic or genetic characteristics or special behavioral or reproductive requirements" (p. 13). Furthermore, this publication also recognizes (p. 13) that; "Population density [crowding] can affect reproduction, metabolism, immune responses, and behavior (Lindsey et al.,[3]." Consequently, this experiment was consistent with NIH guidelines, which recognize and sanction the manipulations of this research methodology (i.e., a special behavioral requirement -- isolation, and different types of crowding).

The housing containers used during the 24-hour housing manipulations were purposely constructed to be of different dimensions. The dimensions of the **spatial** crowded, and **social and spatial** crowded housing containers were 8.6 cms long x 4.5 cms high x 4.1 cms wide. Mice in the **spatial and social** crowded conditions were individually crowded and housed together in one of 10 individual containers placed beside each other in a 2 x 5 arrangement (see Figure 1). Mice in the **social** crowded condition were group crowded and housed together in one container that was 20.0 cms long x 4.5 cms high x 17.5 cms wide. Thus, in all three of the crowded (i.e., **social** crowded, **spatial** crowded, or **spatial and social** crowded) conditions, each mouse had access to the same amount of total space (i.e., total space/number of mice). The dimensions of the **social** crowded container were 10 times as large (i.e., to house 10 mice) as the **spatial, or social and spatial** crowded containers, which housed only 1 mouse. [1] The noncrowded housing containers were the standard, commercially available mouse housing containers described previously.

RESULTS

GENDER EFFECTS

The results of this study demonstrate gender differences associated with the 5 housing conditions and 3 radiation dose levels. A two-way ANOVA between genders and housing conditions within the supralethal 8.8 Gy condition revealed a significant main effect for gender. On the average, female mice lived 20% (+/- 5% longer (mean = 348.7 hours) than did male mice (mean = 290.4 hours) ($F = 19.69$, $df = 1, 135$), $p<.001$.

MALES: HOUSING X DOSE

A two-way ANOVA between the radiation doses and the housing conditions of male mice revealed a significant main effect of dose ($F = 31.21$, $df = 2, 135$), $p<.001$. Male mice in the 8.0 Gy or 8.4 Gy conditions lived an average of 35% (+/- 5%) longer than male mice in the 8.8 Gy condition (see Figure 2). This analysis also revealed a significant main effect of housing. Male mice housed in the **spatial** crowded condition lived on the average 18% (+/- 7%) longer than male mice housed in **isolation.** Male mice housed in the **social** crowded condition lived on the average 23% (+/- 7%) longer than mice housed in **isolation.** Although the average longevity times of male mice between the two lower doses were not significant, different radiosensitivities were noted between housing conditions (Figure 2).

FEMALES: HOUSING X DOSE

A two-way ANOVA between radiation dose and housing of female mice revealed that female mice in the 8.0 Gy condition survived significantly longer than female mice in the 8.4 Gy or 8.8 Gy conditions, except for the one condition noted below (See Figure 3). Newman-Keuls multiple test comparisons revealed the specific differences in longevity of female mice in similar housing conditions across doses of gamma radiation. Across all conditions, only one exception existed to the 8.0 Gy mice surviving significantly longer than mice in

either the 8.4 or 8.8 Gy condition. The survival time of the female mice in the 8.0 Gy isolated housing condition remained significantly ($p<.05$) different from the survival time of female mice in the 8.8 Gy dose, but not from female mice in the 8.4 Gy dose of the same condition.

Further Newman-Keuls multiple test comparisons by housing conditions within the 8.0 Gy dose revealed that female mice in the **social and spatial** crowded condition (mean = 592.8 hours) or **social** crowded (mean = 555.6 hours) conditions survived significantly longer ($p<.05$), than female mice in the **normal housed/social uncrowded** (mean = 466.9 hours), **spatial** crowded (mean = 443.6 hours), or **isolated** (mean = 389.7 hours) condition. The housing effect was so pronounced, that for 60% of the mice in the **social and spatial** crowded condition, and 30% of the mice in the **social** crowded condition, the supra-lethal 8.0 Gy dose was not lethal. In fact, the housing effect of enhanced radioresistance was so pronounced that mice in the **social and spatial** crowded condition lived 34% longer than did mice housed in **isolation.**

DISCUSSION

In order to generalize from animal research to humans, it is important to demonstrate the similarity of responsivity to the same environmental stressors. Studies of humans also show evidence that isolation[7] and crowding [9,57] are stressful life events. Other, human research links stressful life events with immune changes[24]. For example, several researchers have reported an association between the loneliness and "isolation" of spousal bereavement and decreased lymphocyte function[6,24,58,59]. Kiecolt-Glaser et al.,[6] and Hall[24] report that medical students and psychiatric patients that reported a high degree of "isolation" and loneliness exhibited immunosuppression.

The stress resulting from altered housing conditions is viewed here as one of many intraorganism variables moderating the relationship between environmental stimuli and behavioral responses (i.e., longevity). The chain of

events between environmental modifications of housing conditions and behavioral responses contains many intervening links. Previously mentioned animal and human research has demonstrated that these linkages include the neuroendocrine and immune functioning, as well as behavioral well being. The enhanced radioresistance and longevity of mice in certain housing conditions could be due to the nature of the stress-inducing stimuli, being less stressful in some conditions than in other housing manipulations. The selective susceptibility of mice in certain housing conditions to irradiation has contributed to our ever increasing knowledge regarding multiple-stressors (i.e., environmental stimuli and radiation doses) and their powerful effects on longevity.

Just as hardware is tested and optimized for its missions function by careful analysis of weaknesses, so the development of human habitability can be maximized to give the crewmembers the best possible edge on success. The Soviet space program has recognized that habitability issues aboard their space station Mir are directly linked to the success of long duration space flight. Environmental stressors, such as crowding, isolation, and exposure to ionizing radiation will continue to occur in varying amounts and durations in future space exploration. For example, during previous space flights, personnel aboard Skylab 4 and Salyut 6, Expedition IV, received the following dose/dose rates. Skylab personnel (i.e., Carr, Gibson, & Pogue) received approximately 5.03 rads (0.060 RAD/Day)[60,61]. Cosmonauts (i.e., Popov and Ryumin) on Salyut 6, Expedition IV received 2.70 rads (0.015 RAD/Day)[60]. (Note: The original articles reported their findings using rads and no attempt was made to convert their data).

Due to the exotic nature of space flight, occassions will arise where fear is the appropriate response. Fear manipulations are known to be affected by differential social housing in rodents[62] and avians[63]. Crowding is recognized as being an integral part of the spaceshuttle or future space stations[50,51,54]. Isolation will occur during extra-vehicular activity (EVA) (i.e., space walks) or working in a remote area of the space station[53,54,57]. The effects that varying doses of ionizing radiation would have on various housing conditions, including crowding or isolation, across genders, remained largely unknown prior to this experiment. This study has expanded our knowledge, using an animal model,

regarding the types of housing conditions on future space stations. We suggest that future habitability research could benefit by using the same animal model.

SUMMARY

This study examined the longevity of C3H/HeN mice across 5 different housing and three different dose conditions. Previous studies typically examined only 2 altered housing conditions (i.e., isolation or social crowding). These multiple environmental stressors were selected since submariners are, and future space travelers will be, exposed to these housing conditions during their work and rest periods. Since no research existed which addressed these environmental stressors, we sought to quantify the impact of isolation, crowding and ionizing radiation. We have conclusively demonstrated that **environmental manipulations** (i.e., altered housing conditions), for only 24-hours can significantly increase the **radioresistance** of mice exposed to supra-lethal Co-60 gamma radiation.

These dramatic radioresistant effects revealed that a 8.0 supra-lethal Gy exposure of Co-60 was **not** lethal **for 60%** of **social and spatial** or 30% of **social** crowded female mice. Mice in the **social and spatial** crowded condition lived 34% longer than did **isolated** mice and 21% longer than **normally** housed irradiated mice. Also, mice in the **social** crowded condition lived 30% longer than **isolated** mice and 16% longer than **normal housed** mice. The here-to-fore unstudied housing conditions had a dramatic effect on **enhancing** mouse **radioresistance, or mouse radiosensitivity** (i.e., longevity times), even though the mice were handled the same number of times and were only in their unique housing manipulations for **only 24-hours**. In addition, we demonstrated main effects for gender, and dose. Female mice were more **radioresistant** than male mice. A dose effect was also present within genders. 8.0 Gy male mice survived significantly longer than 8.4 Gy or 8.8 Gy male mice and 8.8 Gy female mice lived significantly less than 8.4 Gy or 8.0 Gy female mice. This study extended our knowledge of this species to include supra-lethal Gy doses and multiple housing conditions using survival time as the endpoint. Data indicate the importance of including environmental and biological interaction phenomena in ionizing radiation studies. We have shown the C3H/HEN mouse to be particularly radioresistant or radiosensitive depending upon their housing

condition. Future radiobiology or housing reseaarch would benefit from having the authors clearly state their animal's housing conditions prior to, during, and following their research manipulations.

REFERENCES

1. Brady, J.V., Cohen, H., Dews, P., Fischman, M., Hackman, J.R., Helmrich, R.L., Holloway, H. and Jaffe, J., (1987). Human Behavior, A strategy for space biology and medical science, National Academy Press, Washington, D.C., 165-196.

2. Calhoun, J. (1962). Population density and social pathology, Scientific American, 206, 139-148.

3. Lindsey, J.R., Conner, M.W., and Baker, H.J. (1978). Physical, chemical,, and microbial factors affecting biologic response. Laboratory Animal Housing, National Academy of Sciences, Washington, D.C., 31-43.

4. Worchel, S. and Teddlie, C. (1976). The experience of crowding: A two-factor theory, Journal of Personality and Social Psychology, 34, 30-40.

5. Christian, J.J. and Ratcliff, H.L. (1952). Shock disease in captive wild mammals. American Journal of Pathology, 28, 725-737.

6. Kiecolt-Glaser, J.K., Garner, W., Speicher, C., Penn, G.M., Holliday, J. and Glaser, R. (1984). Psychosocial modifiers of immunocompetence in medical students, Psychosomatic Medicine, 46, 7.

7. Bluth, B.J. (1979). Conscious alteration in space, American Institute od Aeronautics and Astronautics, (AIAA paper 79-1430) Defense Technical Information Center, 79-A34853.

8. Cohen, J.J. (1987). Conference on radiation hormesis: An overview, Health Physics, 52,(5), 519.

9. Baum, A., and Valins, S. (1977). Architecture and social behavior: Psychological studies of social density., Erlbaum, Hillsdale, N.J.:

10. Kirby, J.K., Astor, J., and DelCampo, A.P. (1964). Isolation stress and radiation mortality in rats, Radiation Research, 22, 205.

11. Grahn, D. and Hamilton (1964). Influence of sex, environment, and radiation factors on life shortening and tumor incidence in C3Hf/He mice, Radiation Research, 22, 191.

12. Sacher, G.A. and Grahn, D. (1964). Survival of Mice under duration-of-life exposure to gamma rays: The dosage-survival relation and the lethality function, Journal of the National Cancer Institute, 32,(2), 277-314.

13. Lesher, S.L., Sacher, G.A., Grahn, D., Hamilton, K., and Sallese, A. (1965). Survival of mice under duration-of-life exposure to gamma rays, Radiation Research, 24, 239-277.

14. Grahn, D. (1958). The genetic factor in acute and chronic radiation toxicity. Proceedings, 2nd United Nations International Conference for Peaceful Uses of Atomic Energy, 22, 394-399.

15. Vogel, H.H., Clark, J.W., and Jordan, D.L. (1957). Comparative mortality following single whole-body exposures of mice to fission neutrons and Co 60 gamma rays, Radiation Research, 68, 386-397.

16. Ainsworth, E.J., Leong, G.F., Kendall, K., and Alpen, E.L. (1964). The lethal effects of pulsed neutron or gamma irradiation in mice, Radiation Research, 21, 75-85.

17. Guide for the Care and Use of Laboratory Animals, (1985). National Institute of Health Publication No. 86-23, 11-31.

18. Heiniger, H.J., and Dorey, J.J. (1980). Handbook on genetically standardized JAX mice, 3rd ed., The Jackson Laboratory, Bar Harbor, Maine.

19. Festing, M.F.W. (1979). Inbred strains in biomedical research, New York, Oxford University Press.

20. Vessey, S.H. (1964). Effects of grouping on levels of circulating antibodies in mice, Proceedings of Social and Experimental Biology and Medicine, 115, 252.

21. Edwards, E.A. Rahe, R.H., Stephens, P.M., and Henry, J.P. (1980). Antibody response to bovine serum albumin in mice. The effects of psychsocial environment change. Proceedings of Social and Experimental Biology and Medicine, 164, 478-481.

22. Boranic, M. Poljac-Blazi, M. (1983). Effect of the overcrowding stress on hemopoietic colony formation in mice, Experimental Hematology, 11, 873-877.

23. Coe, C.L., Wiener, S.G., Rosenberg, L.T., and Levine, S. (1985). Endocrine and immune responses to separation and maternal loss in nonhuman primates, In: The Psychobiology of Attachment and Separation, Reite, M.L., and Field, T., (eds.), New York, Academic Press, 1985, 163-200.

24. Hall, S.S. (June, 1989). A molecular code links emotions, mind, and health, Smithsonian, 62-71.

25. Edwards, E.A. Rahe, R.H., Stephens, P.M., and Henry, J.P. (1980). Antibody response to bovine serum albumin in mice. The effects of psychosocial environment change. Proceedings of Social and Experimental Biology and Medicine, 164, 478-481.

26. Kirby, J.K., Astor, J., and DelCampo, A.P. (1964). Isolation stress and radiation mortality in rats, Radiation Research, 22, 205.

27. Rabin, B.S., Lyte, M., Hamill, E. (1987). The influence of mouse strain and housing on the immune system, Journal of Neuroimmunology, 17, 11-16, 1987.

28. Andervont, H.B. (1944). Influence of environment on mammary cancer in mice, Journal of the National Cancer Institute, 4, 579-581.

29. Boranic, M., Polijac-Blazi, M. (1983). Effect of the overcrowding stress on hemopoietic colony formation in mice, Experimental Hematology, 11, 873-877.

30. Rabin, B.S. (1989). The effect of housing (Number of Mice/Cage) on immunologic competency, Office of Naval Research Report, RR04108, 1-6.

31. Landauer, M. (1989). Personal communication.

32. Coe, C.L. Rosenberg, L.t. Fischer, M. and Levine, S. (1987). Psychological factors capable of preventing the inhibition of antibody responses in separated infant monkeys. Child Development, 58, 1420-1430.

33. Coe, C.L., Wiener, S.G., Rosenberg, L.T., and Levine, S. (1985). Endocrine and immune responses to separation and maternal loss in nonhuman primates, In: The psychobiology of Attachment and separation, Reite, M.L., and Field, T., (eds.). N.Y., Academic Press, 163-200.

34. Coe, C.L., Lubach, G.R., Ersher, W.B., and Klopp, R.G. (In Press). Influence of early rearing on lymphocyte proliferation responses in juvenile rhesus monkeys, Brain, Behavior and Immunity.

35. Berkun, M.M., Bialek, H.N., Kern, R.P., and Yagi, K. (1962). Experimental studies of psychological stress in man, Psychological Monographs, 76,(15), Whole No. 534.

36. Brady, J.V. (1984). Human behavior in space environments; A research agenda, The Johns Hopkins University School of Medicine, Baltimore, M.D.

37. Helmrich, R.L., Wilhelm, J.A., and Runge, T.E. (1980). Psychological considerations in future space missions. In T.S. Cheston and D.L. Winter (eds.), Human Factors of Outer Space Production. Westview Press, Boulder, Colorado, 1-23.

38. Kubis, J.F., and McLaughlin, E.J. (Dec, 1967). Psychological aspects of space flight, Transactions of the New York Academy of Science, Series II, 30,(2), 320-330.

39. Levine, A.S. (1965). Prolonged isolation and confinement, Navy - The magazine of sea power, 8,(1), 26-28, 44-45.

40. Bluth, B.J. (Jan, 1982). Staying sane in space, Mechanical Engineering, 104, 24-29.

41. Sexner, J.L. (1968). An experience in submarine psychiatry, American Journal of Psychiatry, 1, 25-30.

42. Vinograd, S.P. (1974). Studies of social group dynamics under isolated conditions, NASA CR-2496, Washington, D.C., 135-146, 214-217.

43. Irwin, M., Daniels, M., Weiner, H. (1987). Immune and neuroendocrine changes during bereavement, Psychiatric Clinic of North America, 10, 449-465.

44. Rabin, B.S., Cunnick, J.E., and Lysle, D.T. (1988). Alterations of the immune system on housing, Advances in Health, 5, 15.

45. Ebersole, J.H. (1960). The new dimensions of submarine medicine, New England Journal of Medicine, 262, 599-610.

46. Weybrew, B.B. (1971). Submarine crew effectiveness during submerged missions of sixty or more days duration, U.S., Naval Submarine Medical Center, Submarine Base, Groton Connecticut, RPT No. 686, 1-22.

47. The Washington Post (1989). Isolation of a cave dweller, p.13.

48. Smith, S. (July, 1969). Studies of small groups in confinement., In: J.P. Zubeck, (ed.), Sensory Deprivation: Fifteen years of research, Appleton-Century-Crofts, N.Y., 374-485.

49. Parker, J.F., and Every, M.G. (1972). Habitability issues in long duration undersea and space missions, Biotechnology, Inc, Falls Church, V.A., 1-52.

50. Stuster, J.W. (Sep, 1986). Space station habitability recommendations based on a systematic comparative analysis of analogous conditions, NASA Report 3943, 94-96.

51. Taylor, T.C., Spencer, J.S., Rocha, C.J., Khan, E., Cliffton, E., Carr, C. (Jan, 1987). Space station architectural elements model study, Nasa Report 4027, 1-140.

52. Nadler, G. (May, 1989). United Press International.

53. Popovich, P.R., and Artiukin, I.U.P. (1979). In: Psychological problems of space flight (A80-32976), 13-53, Moscow, Izdatel'stvo Nauka., 38-41.

54. Collins, D.L. (April, 1985). Psychological issues relevant to long-duration spaceflight: A review of the literature, AFHRL-TP-84-41, National Technical Information Service (NTIS) AD-A154-056, 1-55.

55. Brown, W.R. (Oct, 1983). 1976 Hanford americium exposure incident: Psychological Aspects, Health Physics, 45(4), 867-871.

56. Mickley, G.A. (1979). Psychological effects of nuclear warfare, In: Military Radiobiology, J.J. Conklin and R.I.Walker, (eds.), Academic Press, N.Y., 303-319.

57. Lomov, B.F. (1979). In: Psychological problems of space flight (A80-32976), 13-53, Moscow, Izdatel'stvo Nauka., 38-41.

58. Berkman, L.F., and Syme, L. (1979). Social networks, host resistance, and mortality: A nine-year follow-up study of Alameda County residents, American Journal of Epidemiology, 109, 186-204.

59. Schleifer, S., Keller, S. McKegney, F., Stein, M. (May, 1980). Bereavement and lymphocyte function, paper presented to the American Psychiatric Association Annual Meeting, Montreal, Canada.

60. Vorobyov, Y.I. and Kovalyov, Y.Y. (1983). Radiation of flying crews of flying vehicles, Energoatomizdat, Moscow, Ch.7.

61. English, R.A. (27 Feb, 1974). Radiological health report for SL-4 (R+21 Report), SR-74-6.

62. Suarez, S.D. and Gallup, G.G., Jr. (1981). An ethological analysis of open-field behavior in rats and mice. Learning and Motivation, 12, 342-363.

63. Eddy, T.J. and Gallup, G.G., Jr. (1990). Thermal correlates of tonic immobility and social isolation in chickens, Physiology and Behavior, 47, 641-646.

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1. The minor difference in total area that results if the above dimensions are multiplied is simply due to rounding to the nearest tenth of a decimal place.

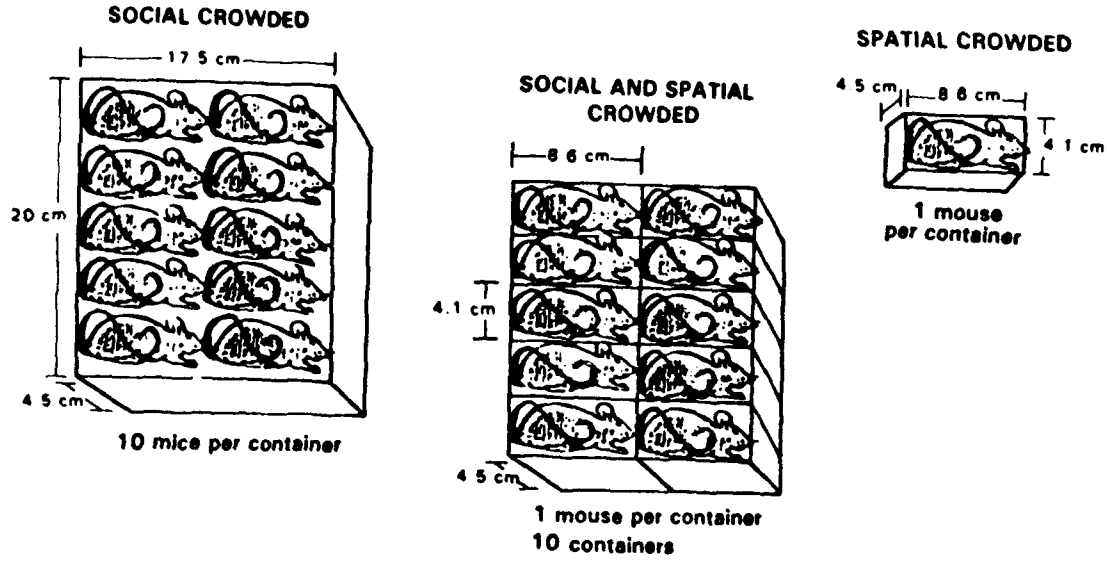
FIGURE 1: This figure displays the five housing conditions in which the mice resided for 24 hours following their ^{60}Cobalt gamma radiation. The housing conditions were: social crowded (n = 10/small cage), spatial crowded, (n = 1/very small cage), social and spatial crowded (n = 1 per very small cage placed flush against each other in a 2 x 5 matrix), spatial uncrowded/isolated (n = 1), or social uncrowded/normal housed (n = 10/cage). Spatial crowding refers to a condition of being constrained due to physical boundaries (i.e., very little space). Social crowding results from an over abundance of animals per unit area. Social and spatial crowding refers to the combination of each of these crowding characteristics. Isolated refers to a housing condition where the mouse is alone in its cage.

FIGURE 2: Survivability of male C3H/HeN mice across 5 different housing (see Figure 1) and 3 different ^{60}Cobalt gamma radiation conditions. Variance indicators are the Standard Error of the Means.

FIGURE 3: Survivability of female C3H/HeN mice across 5 different housing (see Figure 1) and 3 different ^{60}Cobalt gamma radiation conditions. Variance indicators are the Standard Error of the Means.

HOUSING CONDITIONS

CROWDED



UNCROWDED

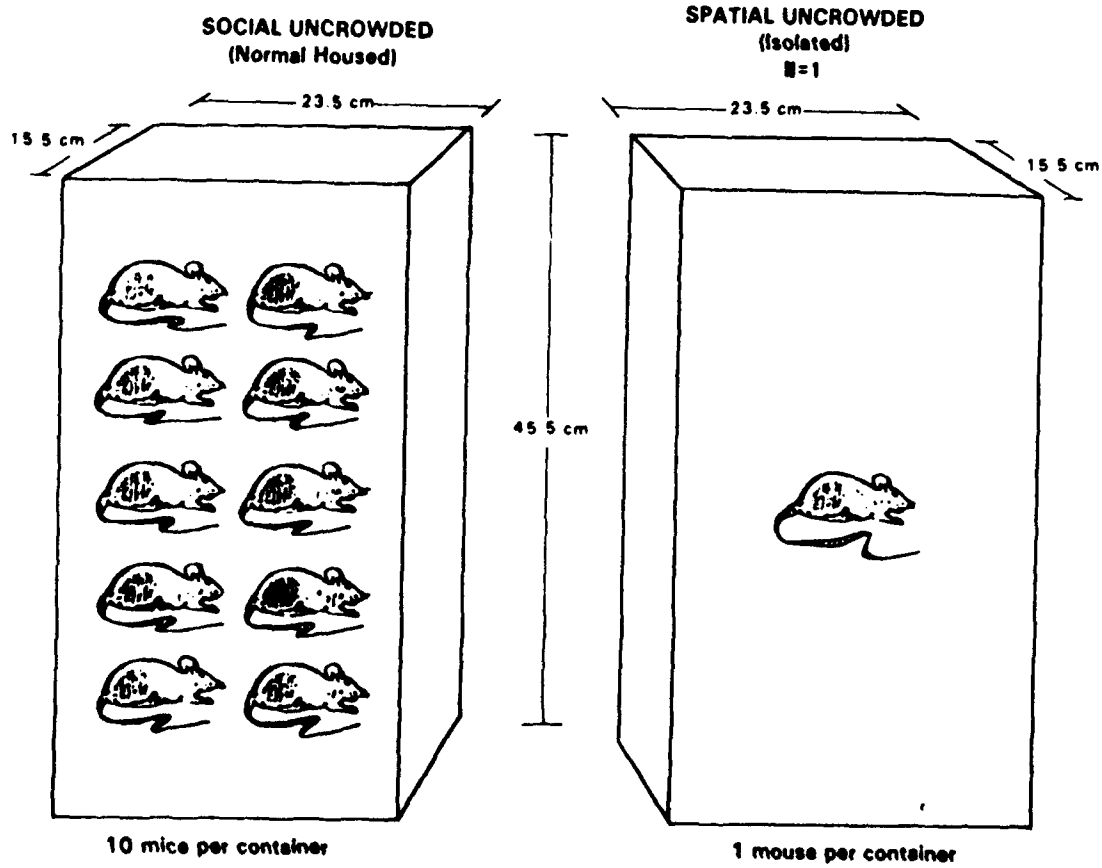


FIGURE 1

FIGURE 2

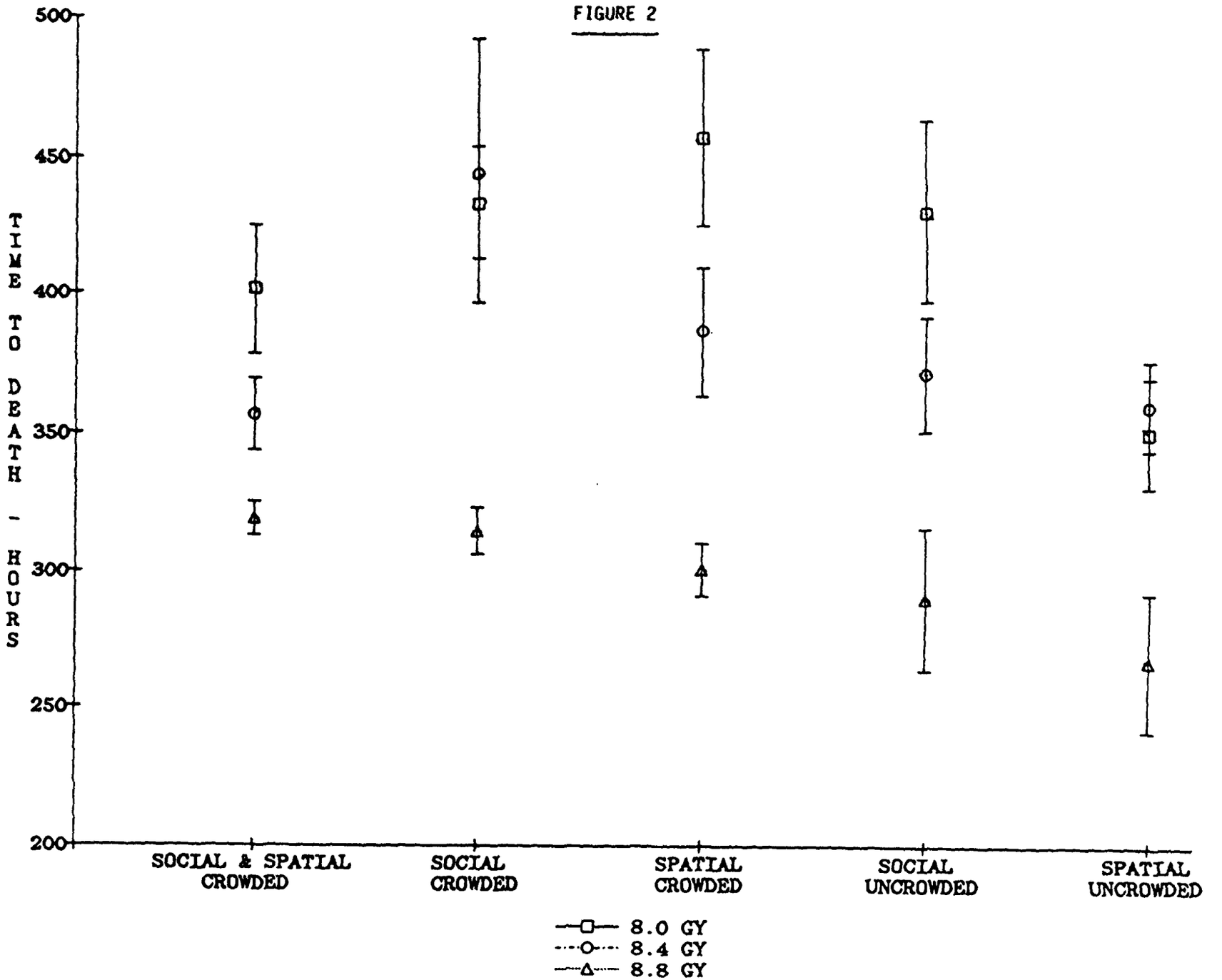
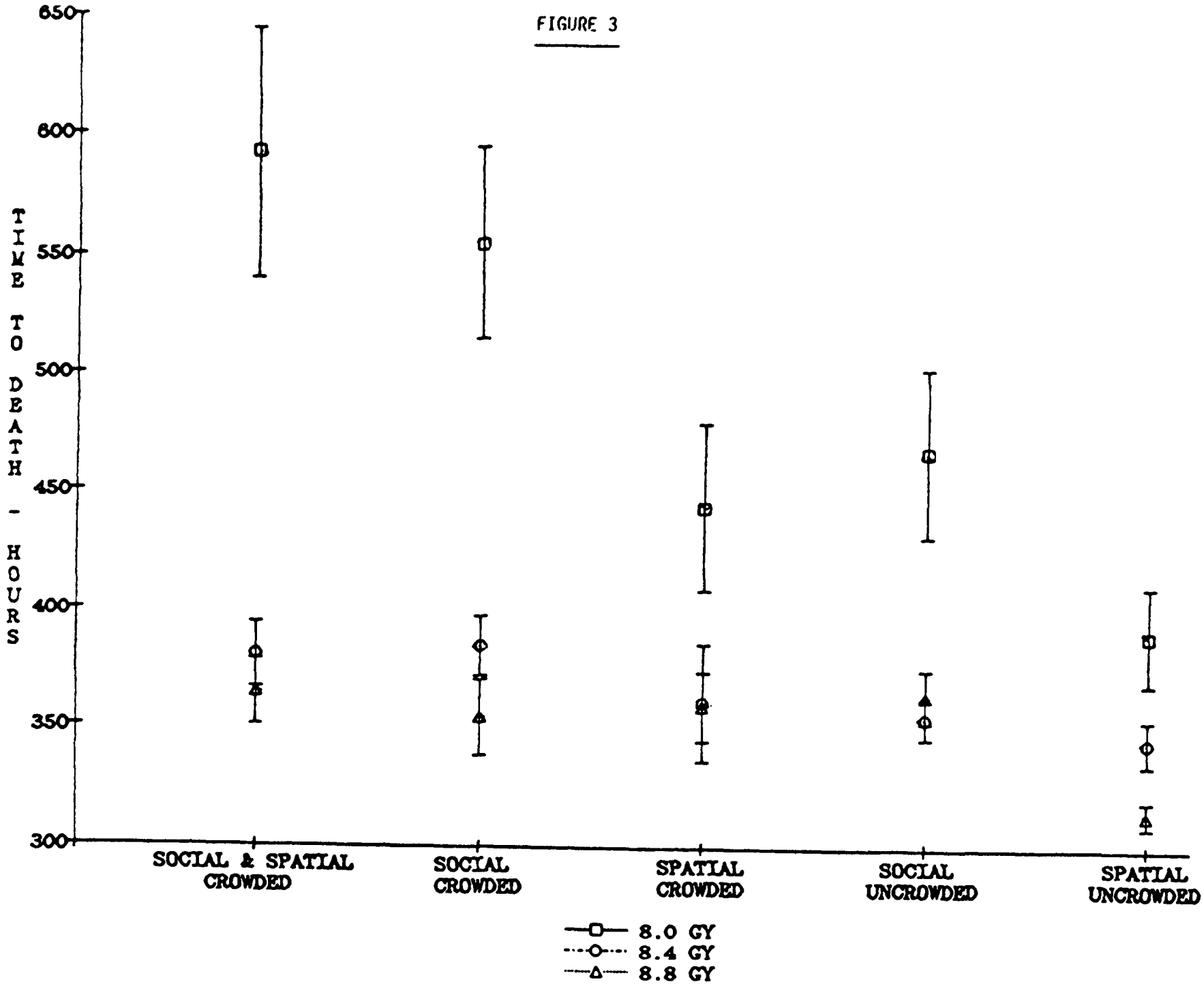

500
450
400
350
300
250
200
TIME TO DEATH - HOURS
SOCIAL & SPATIAL CROWDED
SOCIAL CROWDED
SPATIAL CROWDED
SOCIAL UNCROWDED
SPATIAL UNCROWDED
8.0 GY
8.4 GY
8.8 GY

FIGURE 3


650
600
550
500
450
400
350
300
TIME TO DEATH - HOURS
SOCIAL & SPATIAL CROWDED
SOCIAL CROWDED
SPATIAL CROWDED
SOCIAL UNCROWDED
SPATIAL UNCROWDED
8.0 GY
8.4 GY
8.8 GY